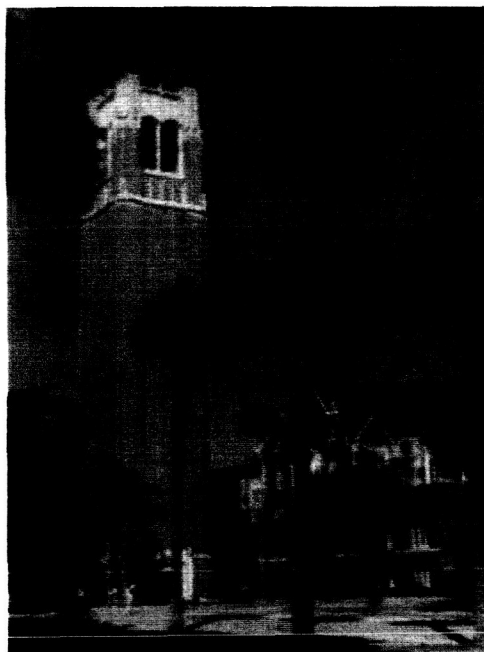


Space Biotechnology and Commercial Applications

University of Florida

NASA KSC Grant NAG 10-316

**Final Report
December 2004**



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Introduction

The Space Biotechnology and Commercial Applications grant was funded by NASA's Kennedy Space Center in FY 2002 to provide dedicated biotechnology and agricultural research focused on the regeneration of space flight environments with direct parallels in Earth-based applications for solving problems in the environment, advances in agricultural science, and other human support issues amenable to targeted biotechnology solutions. This grant had three project areas, each with multiple tasks. They are:

- 1) Space Agriculture and Biotechnology Research and Education,
- 2) Integrated Smart Nanosensors for Space Biotechnology Applications,
- 3) Commercial Applications.

A brief description of each project area is given below:

1. The Space Agriculture and Biotechnology Research and Education (SABRE) Center emphasized the fundamental biology of organisms involved in space flight applications, including those involved in advanced life support environments because of their critical role in the long-term exploration of space. The SABRE Center supports research at the University of Florida and at the Space Life Sciences Laboratory (SLSL) at the Kennedy Space Center. Under the SABRE program, four laboratories and a number of offices in this new facility have been dedicated to University of Florida research needs at SLSL. In 2003, the first full-time UF faculty member was hired as a permanent position located at the SLSL to conduct research under the SABRE program. This position is fully-funded by the State of Florida through the Institute of Food and Agricultural Sciences at the University of Florida.

2. The Integrated Smart Nanosensors for Space Biotechnology Applications component focused on developing and applying sensor technologies to space environments and agricultural systems. The research activities in nanosensors were coordinated with the SABRE portions of this grant and with the research sponsored by the NASA Environmental Systems Commercial Space Technology Center located in the Department of Environmental Engineering Sciences. Initial sensor efforts have focused on air and water quality monitoring essential to humans for living and working permanently in space, an important goal identified in NASA's strategic plan. The closed environment of a spacecraft or planetary base accentuates cause and effect relationships and environmental impacts. The limited available air and water resources emphasize the need for reuse, recycling, and system monitoring. It is essential to collect real-time information from these systems to ensure crew safety. This new class of nanosensors will be critical to monitoring the space flight environment in future NASA space systems.

3. The Commercial Applications component of this program pursued industry partnerships to develop products for terrestrial use of NASA sponsored technologies, and in turn to stimulate growth in the biotechnology industry. For technologies demonstrating near term commercial potential, the objective is to include industry partners on or about the time of proof of concept that will not only co-invest in the technology but also take the resultant technology to the commercial market. In this manner, the grant funding is leveraged against commercial funding from industry partnerships; UF has potential for increased licensing and royalty income; and NASA is credited with providing commercial products derived from its sponsored research. The commercial applications process conducts commercial market assessments on emerging technologies, addresses and resolves the intellectual property rights of the potential team members, and develops collaborative industry partnerships for the mutual benefit of UF, NASA, and the private sector.

Space Agriculture and Biotechnology Research and Education (SABRE)

Principal Investigator: Dr. Robert Ferl

For most of the project effort, the science outcomes were presented in the 2003 Final Report documents. However, there were several projects that continued significant activity and findings during the extension year. In addition, two new research projects and infrastructure elements were completed.

Project Continuations:

I. Development of a genetics-based computer model for the engineering of new crops

Eduardo Vallejos, James W. Jones

The project goals were too modify the BEANGRO program to include genetic coefficients for *state variables*. The genetic-based program will be adjusted and tested for its phenotype predicting ability of specific allele combinations. The new program will help in the design of new specialized crops.

The BEANGRO model was modified to include some specific genes (Fig 1). The modified model can predict, to some extent, yield and seed size characteristics. However, these results highlight the need to identify and add new genetic factors to the model to improve its performance. These data were obtained from several bean genotypes. In addition, we have collected field and greenhouse data on seed size (yield component), growth habit and phenology of a recombinant inbred family of the common bean, *Phaseolus vulgaris*. Model performance can be improved with data obtained under different photoperiod and temperature combinations. We are planning to do this in collaboration with the International Center for Tropical Agriculture (CIAT) in Cali, Colombia. A proposal with Dr. S. Beebe (CIAT) as collaborator was submitted to NSF.

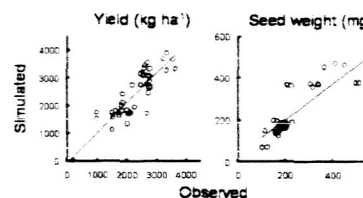


Figure 1. Response of Beangro after incorporation of genetic coefficients for Ppd, Hr, Fin, Fd, Ssz1, Ssz2 and Ssz3.

To facilitate marker analysis of additional RI lines, which will be added to the current 76 lines, we have converted 210 RFLP markers into PCR-based markers. These genomic clones were first sequenced, and the sequences were analyzed to design primers for PCR amplification. While RFLP probes can detect DNA polymorphism within and far beyond the sequence they represent, PCR-based markers can only detect polymorphism within the stretch of DNA flanked by the PCR primers. To increase the likelihood of detecting polymorphisms, PCR primers were designed from conserved exon regions, and at least one intron was included in the amplicon.

Exon-intron junctions were deduced from a combination of BLASTX searches, and analysis with SplicePredictor, a WEB-based utility designed by V. Brendel at Iowa State University. Identification of significant sequence similarities between our probes with GeneBank entries will facilitate establishing syntenic relationships with other legumes, and increase the probability of extending these findings to other related crops.

Grant Proposal Submitted to NSF

“A Computational Systems Biology Approach for Evaluating and Utilizing QTL Data.”

PIs: CE Vallejos, JW Jones, R Wu and CD Messina. Status: Pending.

Oral Presentation

CD Messina, CE Vallejos, JW Jones. 2004. Linking Biophysical Models, Bean Genomics and Optimization Algorithms for Plant Ideotype Design in Life Support Systems. Meeting: Habitat 2004, January 7, 2004, Orlando, FL.

Manuscript in Preparation

Vallejos CE, CD Messina and JW Jones. Genetic analysis of seed size in the common bean, *Phaseolus vulgaris*.

Vallejos CE. Sequence analysis and conversion of RFLP markers to PCR-based markers in the common bean, *Phaseolus vulgaris*.

II. Metabolomics of temperature shock

Charles Guy, Fatma Kaplan

Metabolic profiling analyses were performed to determine metabolite temporal dynamics associated with the induction of acquired thermotolerance in response to heat shock and acquired freezing tolerance in response to cold shock. Low molecular weight polar metabolite analyses were performed using gas chromatography and mass spectrometry (GC-MS). Eighty-one identified metabolites and 416 unidentified mass spectral tags (MSTs), characterized by retention time indices and specific mass fragments, were monitored. Cold shock influenced metabolism far more profoundly than heat shock. The steady-state pool sizes of 143 and 311 metabolites or MSTs were altered in response to heat and cold shock, respectively. Comparison of heat and cold shock response patterns revealed that the majority of heat shock responses were shared with cold shock responses, a previously unknown relationship. Coordinate increases in the pool sizes of amino acids derived from pyruvate and oxaloacetate, polyamine precursors and compatible solutes were observed during both heat and cold shock. In addition, many of the metabolites that showed increases in response to both heat and cold shock in this study were previously unlinked with temperature stress. This investigation provides new insight into the mechanisms of plant adaptation to thermal stress at the metabolite level, reveals relationships between heat and cold shock responses, and highlights the roles of known signaling molecules and protectants.

Grants submitted based on project funding:

CDDF: A Focused Metabolite Profiling Capability for Dissecting Cellular and Molecular Processes of Living Organisms in Space Environments. Submitted by Lanfang Levine, Charles Guy, John Sager and Ray Wheeler. A Center Director's Discretionary Fund Proposal for 2 years. Proposal was recently funded.

Papers based on project funding:

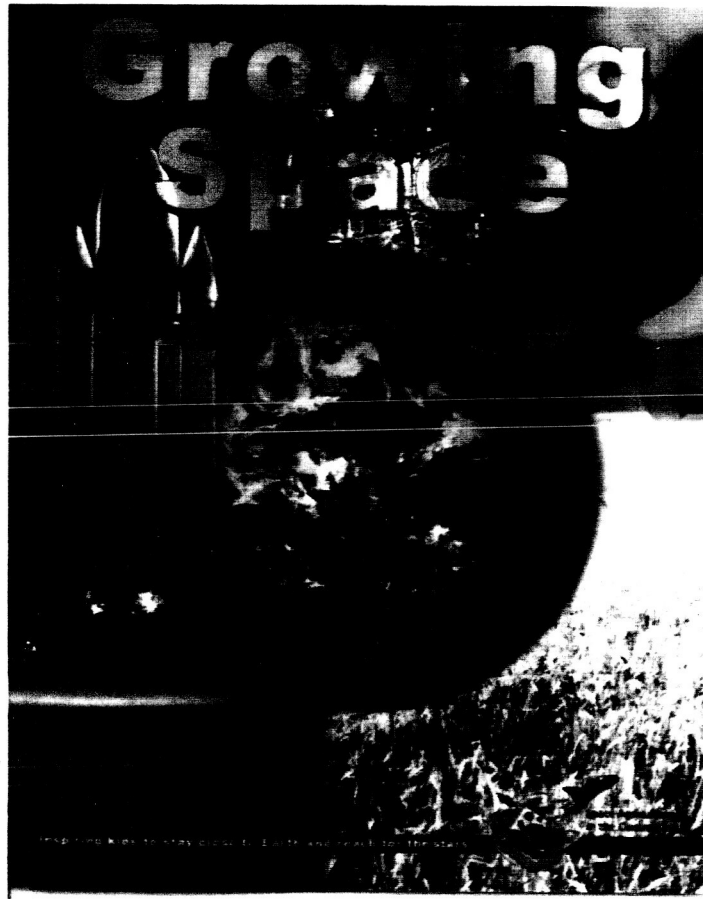
Exploring The Temperature Stress Metabolome Of Arabidopsis. Fatma Kaplan, Joachim Kopka, Dale W. Haskell, Wei Zhao, K. Cameron Schiller, Nicole Gatzke, Dong Yul Sung, Charles L. Guy Plant Physiology (accepted).

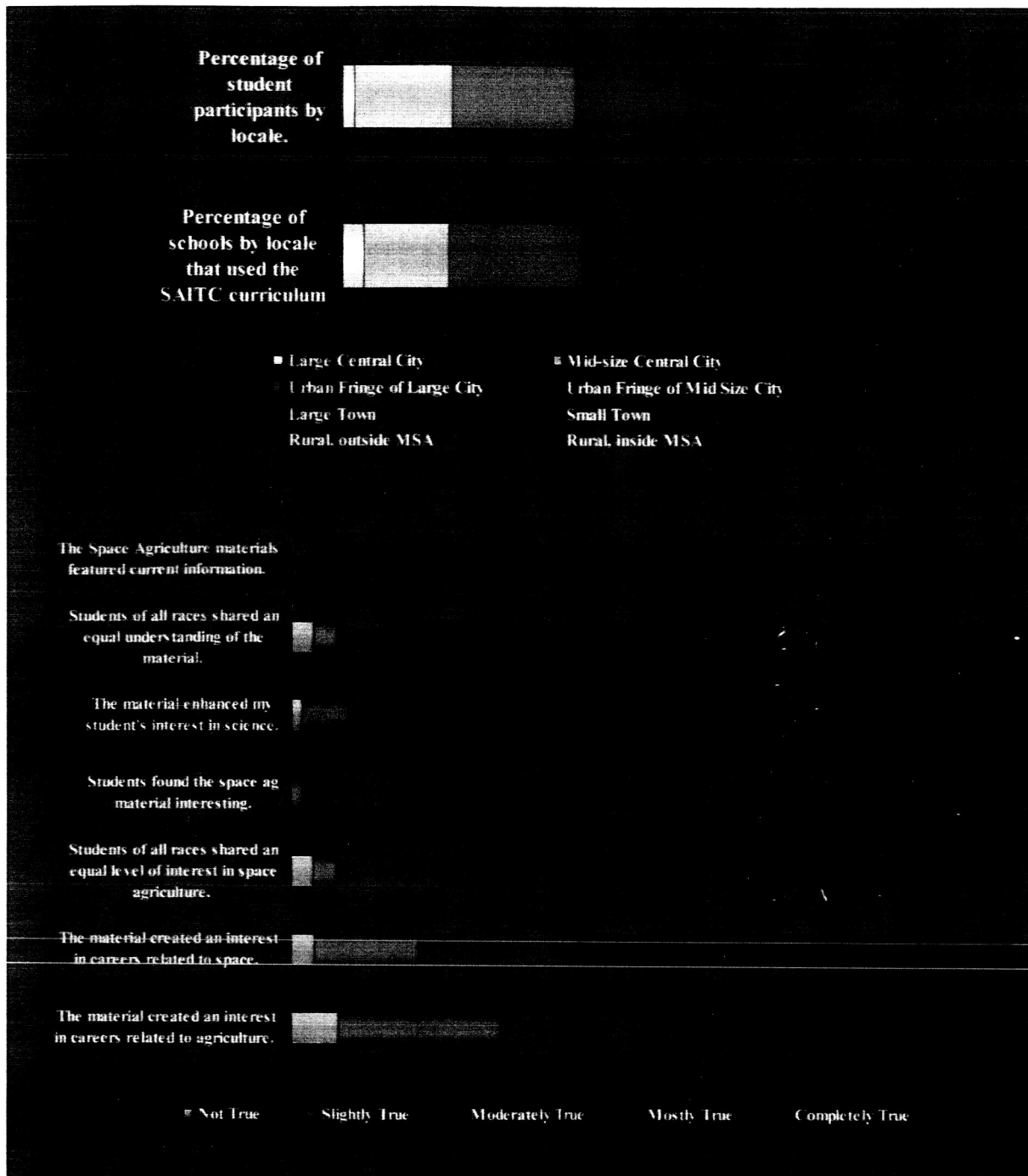
Ethanollic Fermentation During Cold Shock: A Novel Plant Temperature Stress Coping Strategy. (In preparation).

III. Space Ag In The Classroom

Ed Osbone, Jim Dyer, Glenn Israel, Shannon Washburn

The Space Ag In The Classroom project completed its study of the programs participation and impact. The goal of the project was to develop educational materials based upon *Farming in Space*, *BioBLAST*, and similar curricula for dissemination to approximately 50,000 sixth grade science students in four states. Curricular focus will be on earth-based agricultural applications of space-based research, particularly in the plant sciences. Anticipated outcomes included increased appreciation for the benefits of space life sciences research and greater understanding and awareness of the agricultural industry. Some of the results include the following data:





These additional participation data are being summarized for publication.

Project Publications

Washburn, S., Dyer, J., Dragon, S., Osborne, E., Israel, G. & Park, L. (2003). *Growing Space*, a 12-page, four-color educational magazine developed for middle school science students. (55,000 copies printed)

Rhoades, E., Osborne, E., Dyer, J., Washburn, S., Israel, G., & Park, L. (2003). *Space Agriculture in the Classroom Project Website*, available at www.spaceag.org.

Bellah, K., Washburn, S., Dyer, J., Osborne, E., Israel, G., Park, L., Toro, E., & Rhoades, E. (2003, December). *Space Agriculture in the Classroom*. Refereed poster accepted for presentation at the American Association for Agricultural Education annual conference, Orlando, FL.

Bellah, K., Washburn, S., Dyer, J., Osborne, E., Israel, G., Park, L., Toro, E., & Rhoades, E. (2004, January). *Space Agriculture in the Classroom*. Refereed poster proposal submitted for presentation at the 2003 Habitation Conference, Orlando, FL (under review).

Dyer, J., Bellah, K., Osborne, E., Washburn, S., & Israel, G. (2004, June). *Feeding the Astronauts – Space Agriculture in the Classroom*. Refereed workshop accepted for presentation at the National Agriculture in the Classroom Conference, Albuquerque, NM

New Project Completions:

I. Mars Astrobiology

The laboratory of Dr. Andrew Scheuerger completed set up at the SLS Laboratory at KSC, in part with a subaccount grant from this project.

A) Grants derived in part from the project:

UCF/UF Space Research Initiative Grant (#20020023/21988; jointly funded by University of Central Florida and University of Florida); "Survival, Ecology, and Detection of Endolithic Microbial Communities under Simulated Martian Environmental Conditions." Grant value: \$210K for 2004.

Research: The collaborators on this project are Dan Britt (UCF), Laura Woodney (UCF), and Wayne Nicholson (UF). The project will study the effects of Martian conditions on (i) the remote sensing of Mars analog rocks and soils, (ii) the detection of endolithic microbial communities in Mars analog rocks, (iii) the growth of terrestrial bacteria within Mars analog rocks, and (iv) the internal atmospheric conditions within the void spaces of Mars analog rocks and soils. **Results** will help constrain the search for life on Mars and may contribute to the qualitative and quantitative characterization Mars rocks and soils as imaged from the Mars rovers Spirit and Opportunity.

JPL/NASA (#JPL-GJT-569274); "Effects of Diffuse UV irradiation on the Survival of Terrestrial Bacteria on Spacecraft Components." Grant Value: \$115 K for FY2002 – FY2004.

Research: A collaborative project is underway with Peter Smith and Roger Tanner (U. of Arizona) and Charles Cockell (British Antarctic Survey) to study the effects of diffuse UV irradiation on the survival of terrestrial bacteria on

spacecraft components. **Results** have suggested that the combined direct and diffuse UV levels on Mars (190-320 nm) will provide adequate UV irradiation to inactivate most common terrestrial bacteria found on spacecraft surfaces. Full biocidal activity of most microbial species will be achieved within seconds to minutes on Mars independent of latitudinal position of the landers, solar zenith angles, or optical depths of the Martian atmosphere.

NASA Research Grant (#ROSS-99 NRA 99-OSS-01); "Survival of Terrestrial Microorganisms on Spacecraft Components and Analog Mars Soils under Simulated Martian Conditions." Grant Value: \$ 414 K for FY2000 – FY2004.

Research: A collaborative project is underway with Rocco Mancinelli, Lynn Rothschild, Chris McKay (all from NASA/Ames Research Center), and Roger Kern (NASA/JPL) to study the survival of terrestrial microorganism on spacecraft materials under simulated Martian conditions. Experimental conditions have included Mars-normal atmospheric pressure and gas composition, diurnal temperature fluctuations, and the UV, VIS, and NIR fluence rates on the surface estimated for an equatorial lander mission. Experiments were conducted within a Mars Simulation Chamber at the Kennedy Space Center, FL. The MSC is a low-pressure chamber in which Mars-normal conditions of gas composition, temperature, pressure, and UV irradiation can be precisely controlled for any location on the surface of the planet. **Results** indicated that gas composition and temperature have no effect, and low pressure only a minor effect, on bacterial survival under simulated Martian conditions. Greater than 99% of the biocidal activity on the surface of Mars is due to UV irradiation.

B) New 2004 Proposals derived in part from the project:

- 1) *Spectral Reflectance of Analog Mars Rocks, Soils, and Endolithic Microbial Communities Exposed to Martian Environmental Conditions.* Submitted to Mars Fundamental Research (ROSS #NNH04ZSS001N-MFRP); research team: Dr. **Andrew Schuerger, PI** (UF); Dr. Dan Britt, Co-I (UCF), Dr. Laura Woodney, Co-I (UCF); amount requested = \$306,280 over 3 yrs.
- 2) *Spectral Reflectance of Analog Mars Rocks, Soils, and Endolithic Microbial Communities Exposed to Martian Environmental Conditions.* Submitted to NASA's Planetary Protection Office (ROSS #NNH04ZSS001N-PPP); research team: Dr. **Andrew Schuerger, PI** (UF) and Dr. Wayne Nicholson, Co-I (UF); amount requested = \$323,989 over 3 yrs.
- 3) *Effects of Soil Chemical Factors on the Growth of Plants in Mars Analog Soils under Simulated Mars Lander Experimental Conditions.* Submitted to the Office of Biological and Physical Research (OBPR #NNH04ZUU004N); research team: Dr. **Andrew Schuerger, PI** (UF), Dr. Horton Newsom, Co-I (UNM), Dr. Doug Ming, Co-I (JSC), Dr. Rob Ferl, Co-I (UF), and Dr. Anna-Lisa Paul, Co-I (UF); amount requested = \$585,411 over 3 yrs.
- 4) *Role of Space Radiation on the Survival, Mutagenesis, and Future Adaptation of Terrestrial Microorganisms on Spacecraft Headed for Mars.* Submitted to NASA call for Ground-Based Studies for Radiation Biology and Radiation Shielding Materials: research team: Dr. **Andrew Schuerger, PI** (UF), Dr. Wayne Nicholson, Co-I (UF), and Dr. Marcelo Vazquez, Co-I (Brookhaven Labs, NY); amount requested \$1.2 million over 4 yrs.

- 5) *Degradation of Biological Signature Molecules in Analogue Martian Environments*. Submitted to NASA's Planetary Protection Office (ROSS #NNH04ZSS001N-PPP); research team: Dr. Wayne Nicholson, PI (UF) and Dr. **Andrew Schuerger**, Co-I (UF); amount requested = \$325,560 over 3 yrs.
- 6) *Glow Discharge on Mars: Potential Factors in the Degradation of Organics in the Martian Regolith*. Submitted to Mars Fundamental Research (ROSS #NNH04ZSS001N-MFRP); research team: Dr. Carlos Calle, PI (KSC), Dr. Charles Buhler, Co-I (KSC), Dr. **Andrew Schuerger**, Co-I (UF); amount requested = \$378,710 over 3 yrs.
- 7) *Bulge-Test Apparatus for Studying the Combination of Stress and Environmental Effects on Polymer Films*. Phase I study submitted to the NASA SBIR program; research team: Mr. Jim Clawson, PI (UC in Boulder, CO), Dr. **Andrew Schuerger**, Co-I (UF), amount requested = \$75,000 for 6-months.
- 8) *An Expert System for Monitoring Plant Health in Bioregenerative Life Support Modules*. Phase I study submitted to the NASA SBIR program; research team: Mr. Mathew Bethel, PI (Photon Industries, LA), Dr. **Andrew Schuerger**, Co-I (UF), Dr. Mark Lewis (Photon Industries); amount requested = \$75,000 for 6-months.

C) Publications related to project:

- 1) Cockell, C. S., and Schuerger, A. C. 2004. Life and death of an extremophilic photoautotroph (*Chroococcidiopsis* sp. 029) under a simulated Martian UV flux. *Astrobiology* (in press).
- 2) Newcombe, D. A., Schuerger, A. C., Bernardini, J. M., Dickinson, D., Tanner, R., and Venkateswaran, K. 2004. Survival of spacecraft associated microbes under simulated Martian ultraviolet irradiation. *Applied Environ. Microbiol.* (submitted).
- 3) Nicholson, W. L., Schuerger, A. C., and Setlow, P. 2004. The Solar UV Environment and Bacterial Spore UV Resistance: Considerations for Panspermia and Planetary Protection. *Mutation Research* (in press).
- 4) Paul, A-L., Schuerger, A. C., Popp, M., Richards, J. T., Manak, M., and Ferl, R. J. 2004. Hypobaric biology: Arabidopsis gene expression at low atmospheric pressure. *Plant Physiology* 134:215-223.
- 5) Richards, J. T., Corey, K., Paul, A-L., and Ferl R.J., and Schuerger, A. C. Short term exposure of *Arabidopsis thaliana* to low-atmospheric pressure environments: Effects on CO₂ gas exchange characteristics and transpiration rates. *Astrobiology* (submitted)
- 6) Schuerger, A. C., Mancinelli, R. L., Kern, R.G., Rothschild, L. J., and McKay, C P. 2003. Survival of *Bacillus subtilis* on spacecraft surfaces under simulated Martian environments: Implications for the forward contamination of Mars. *Icarus* 165(2):253-276.
- 7) Schuerger, A. C., Richards, J. T., Hintze, P. E., and Kern, R. Surface Characteristics of Spacecraft Components Affect the Aggregation of Microorganisms and May Lead to Different Survival Rates of Bacteria on Mars Landers. *Astrobiology* (submitted).
- 8) Schuerger, A. C., Richards, J. T., Newcombe, D. A., and Venkateswaran, K. J. 2004. Survival of seven *Bacillus* spp. under simulated Mars UV irradiation suggests minimum forward contamination around landing sites. *Icarus* (in preparation).

II. Interplanetary Biological Transfer

The laboratory of Dr. Wayne Nicholson completed set up at the SLS Laboratory at KSC, in part with a subaccount grant from this project.

A) Current grant support benefiting from the current project:

As P.I.:

NASA Exobiology (NCC2-1342). "Survival of endolithic bacteria to impact-generated launch conditions". [REDACTED] direct, [REDACTED] total. 6/1/02-5/31/04. (Transferred from U. Arizona to U. Florida to become:)

NASA Exobiology (NNA04CI35A). "Survival of endolithic bacteria to impact-generated launch conditions", [REDACTED] total.

USDA-Hatch, Florida Agricultural Experimental Station (MCS-04164). "Resistance of sporeforming soil bacteria to UV radiation", 11/1/03-10/31/04.

As Co-I:

UCF-UF Space Research Institute Grant (20020023/21988): "Survival, Ecology, and Detection of Endolithic Microbial Communities under Simulated Martian Environmental Conditions". PI: Andrew Schuerger. 11/30/03-6/30/04 Total cost: [REDACTED]. UF Portion: [REDACTED] UCF Portion: \$ [REDACTED] Co-I portion: [REDACTED]

NSF-DDIG. "Evolution of Genomic Architecture in Laboratory Populations of *Bacillus subtilis*", 7/1/04-6/30/06. [REDACTED]. Doctoral dissertation support for grad. Student Heather Maughan.

NSF-USDA "Genome sequencing of *Bacillus pumilus*" 9/1/04-8/31/06. [REDACTED] direct costs. PI: George Weinstock, Human Genome Sequencing Center, Baylor College of Medicine.

B) Pending Grant Proposals derived in part from the project:

NASA (PI) "Degradation of biological signature molecules in analogue Martian environments" 01/01/2005-12/31/2007. Total Proposed Cost: [REDACTED]

NASA (PI) " Genomic and phenotypic changes during long-term bacterial evolution in human advanced life support habitats". 01/01/2005-12/31/2007. Total Proposed Cost: [REDACTED]

NASA-ESA (Co-I) "PROTECT: Resistance of spacecraft isolates to outer space for planetary protection purposes" 3 years, [REDACTED] total for my part. PI: Gerda Horneck, DLR, Köln.

NASA (Co-I) "Low-Pressure Microbiology: The Study of Microbial Metabolism, Growth, and Replication under Martian Conditions" (A. Schuerger, PI). Project Period: January 1, 2005 – December 31, 2007. Total Proposed Cost: [REDACTED]

C) Publications related to the project:

Nicholson, W.L., A.C. Schuerger, and P. Setlow. 2004. The solar UV environment and bacterial spore UV resistance: considerations for Earth-to-Mars transport by natural processes and human spaceflight. *Mutat. Res.* (in press).

Fajardo-Cavazos, P., L. Link, H.J. Melosh, and W.L. **Nicholson.** 2004. *Bacillus subtilis* spores in simulated meteorite survive hypervelocity atmospheric entry: implications for lithopanspermia (in preparation).

Nicholson, W.L., P. Fajardo-Cavazos, and H.J. Melosh. 2004. Endolithic *Bacillus subtilis* spores survive launch driven by hypervelocity impact (in preparation).

Nicholson, W.L. and A.C. Schuerger. 2004. Survival and germinability of *Bacillus subtilis* spores after incubation at Mars atmospheric pressure: implications for planetary protection and lithopanspermia (in preparation).

Abstracts:

Nicholson, W.L. 2004. Astromicrobiology: introduction to microbiology in the space environment. Abstracts of 10th International Symposium on Microbial Ecology (ISME-10), Cancun, Mexico, p. 360.

Fajardo-Cavazos, P., L. Link, H.J. Melosh, and W.L. **Nicholson.** 2004. Testing lithopanspermia theory: survival of bacterial spores in granite to forces generated during impact-driven launch and high-velocity atmospheric entry. Abstracts of the 10th International Symposium on Microbial Ecology (ISME-10), Cancun, Mexico, p.74.

Kamine, T.H., P. Fajardo-Cavazos, and W.L. **Nicholson.** 2004. Survivability of *Bacillus subtilis* Spores After Impact-Mediated Ejection from a Planet. American Society for Gravitational and Space Biology, Student Poster Competition (submitted)

Invited Platform Talks at Symposia:

"Astromicrobiology: introduction to microbiology in the space environment", in Session "Microbial Ecology of the Space Environment", 10th International Symposium on Microbial Ecology (ISME-10), Cancun, Mexico, August 27, 2004.

Infrastructure Elements

A new growth chamber was added to the Phytotron facility at the SLS Laboratory at KSC. The growth chamber was purchased and installed by UF with the cooperation and support of the LSSC. While primarily for UF use at SLSL, the chamber will be available to support flight and ground experimentation as part of the Phytotron facility.

Integrated Smart Nanosensors for Space Biotechnology Applications

Project Investigator: Dr. Toshikazu Nishida

Integrated Smart Nanosensors

This document provides an update to the final report submitted in November 2003 for the Integrated Smart Nanosensors program in the NASA Space Biotechnology grant centered at the University of Florida. Major expected outcomes of the NASA Space Biotechnology grant at the University of Florida and participating universities are (1) technical advances and (2) seeding further research to benefit NASA mission goals and national technical priorities.

The seed effect of the NASA funding of Integrated Smart Nanosensors is itemized in Section II. Updates on technical advances beyond that presented in the November 2003 final report are discussed in Section III.

Table 1 reviews the organization of the sensor research in three thrust areas: (1) test bed development, (2) miniaturization of existing macro sensors, and (3) novel nanosensor development, targeted towards the development of low mass, low power sensors for NASA Advanced Life Support (ALS) mission goals.

Table 1. Sensor research tasks in the Integrated Smart Nanosensors program.

<i>Test Bed Development</i> Water Purification: Mazyck (EES)
<i>Miniaturization of Macro Sensors</i> Cumulative Flow: Hatfield (CE), Annable(EES) Gas Sensor: Wachsman (MSE)
<i>Nanosensor Development</i> Flow Sensor: Sheplak (MAE) and Nishida (ECE) Wide Band gap Sensors: Ren (CHE), Chauhan (CHE), Pearton (MSE) Membrane Development: Jones (MSE), Law(ECE) Intersensor communication strategies: O (ECE), Sheplak (MAE) Development of disposable microbial sensor for water quality monitoring: Cho (UCF) Cell culture lab-on-a-chip in microgravity: Tran-Son-Tay (MAE) Integration of regenerative enhanced nano-sensors: Wu (EES) Self-Powered Sensors: Nishida (ECE), Sheplak (MAE)

Seed Effect of NASA Funding

The NASA investment in Integrated Smart Nanosensors has seeded [REDACTED] in new funding at the University of Florida ([REDACTED]) and the University of Central Florida ([REDACTED]) which has continued the research efforts on smart integrated nanosensors beyond the completion of the NASA Space Biotechnology project. The funded projects are listed below.

Hyoungh Jin Cho: "CAREER: A Micro SPR (Surface Plasmon Resonance) Sensor with Integrated Microfluidic Components for In-situ Monitoring of Biomolecular Activities", National Science Foundation, 2004-2009, [REDACTED], Funded.

Khai Ngo, "Power for Wireless Hydrogen Sensor Network," NASA Hydrogen Research for Aviation and Space-related Applications, 2004-2005, 1 year, [REDACTED], Funded.

Toshi Nishida, "Power for Wireless Hydrogen Sensor Network—Energy Harvesters," NASA Hydrogen Research for Aviation and Space-related Applications, 2004-2005, 1 year, [REDACTED], Funded.

David Norton, Stephen Pearnton, and Fan Ren, "ZnO PN Junctions for Highly-Efficient, Low-Cost Light Emitting Diodes," DOE Office of Energy Efficiency and Renewable Energy, 2004-2007, 3 years, [REDACTED], Funded.

Ken O, "A Feasibility Study for Wireless Data Communication between Silicon Integrated Circuits using Integrated Antennas," NSF, 2004-2005, 1 year, [REDACTED], Funded.

Mark Sheplak, Lou Cattafesta, Toshi Nishida, "Moire-based optical MEMS shear stress sensor technology," Office of Naval Research, 2004-2007, 3 year, [REDACTED], Funded.

Wolfgang Sigmund et al., "Interdisciplinary Center Planning Grant on Diagnosis and Therapy of Drug and Chemical Toxicity," National Institute of Health, 2004-2007, 3 year, [REDACTED], Funded.

Technical Advances—Updated Highlights

Updated Highlights:

Two projects are highlighted in this no-cost extension period of the Integrated Smart Nanosensors program.

Integration of Regenerative Enhanced Nano-Sensors (IRENS)

C.Y. Wu and W. M. Sigmund (University of Florida)

The goal of this project is to synthesize nanocomposite materials with multiple functions: photocatalytic activity, regeneration, magnetically controlled movement, adsorption and enhanced Raman sensing for contaminant sensing, efficient water treatment, low equivalent systems mass and easy regeneration in microgravity environment. The multifunctional particle is illustrated in Figure 1.

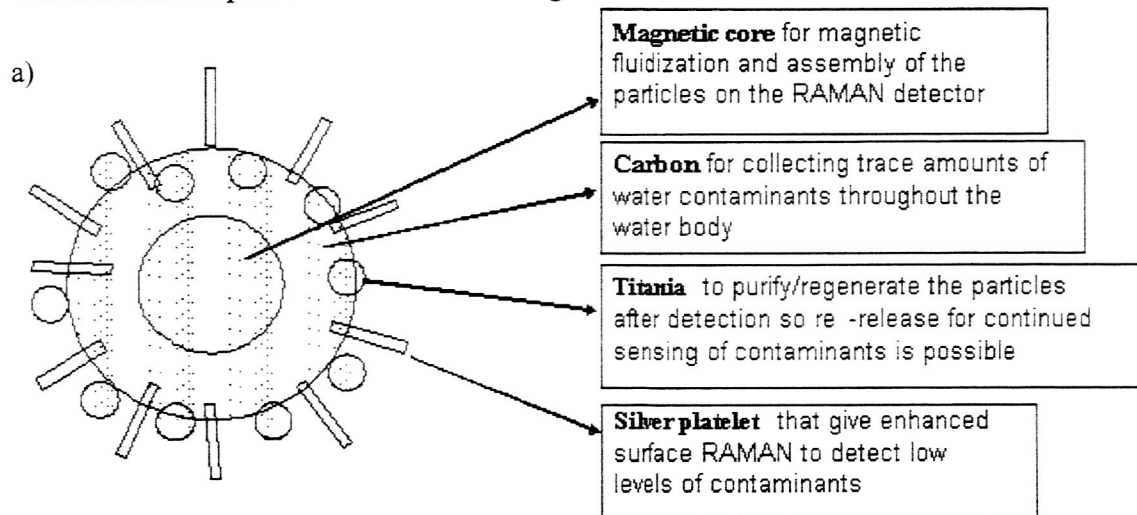


Figure 1. Conceptual drawing of multifunctional particle for water recovery systems.

Figure 2 shows a TEM micrograph of permanent magnet coated with protective silica nano-coating and nanostructured titania photocatalyst. Arrows indicate the silica layer between the magnet and photocatalyst. By combining the magnet with photocatalyst, the movement of the photocatalyst can be controlled in microgravity environment.

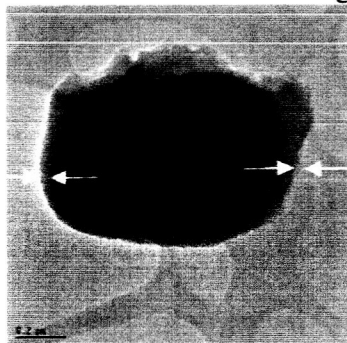


Figure 2 TEM micrograph of B/S/T

Figure 3 shows the HR TEM image of our MWNT-TiO₂ nanocomposite.

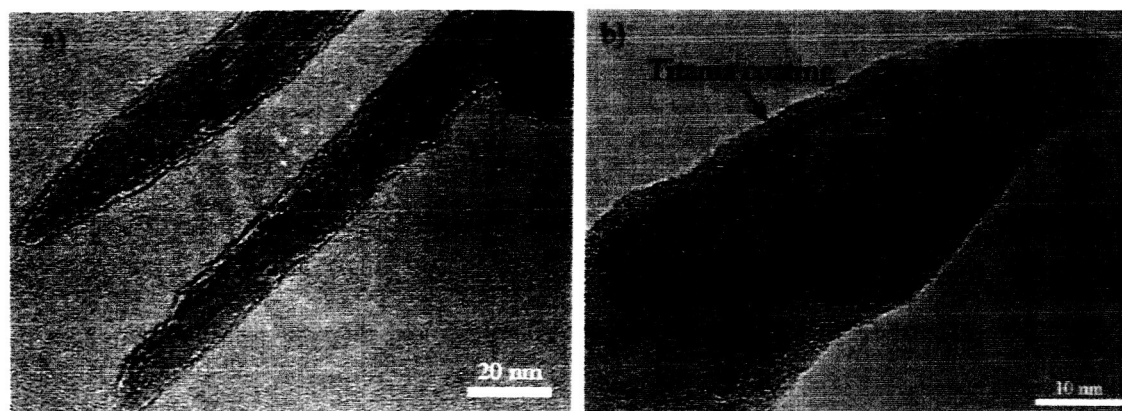


Figure 3. (a) HR TEM image MWNT-TiO₂ nanocomposite. (b) Titania coating.

Table 2 shows the bacterial spore deactivation tests of our MWNT-TiO₂ nanocomposite with benchmarking P25 Titania (Degussa). The performance is twice better than the P25. The reason for the superior performance is due to the modification of Titania's electronic properties. The benchmarking is performed on equal surface area basis. Despite the fact that this reduces the titania by weight to 200 times less than the Degussa P25 the novel material has a performance twice better than the P25.

Table 2 Effect of commercially available TiO₂ particles and TiO₂ – MWNTs nanocomposites under the presence of solar UV on *B. cereus* spores (Biocidal tests were repeated three times)

System	LD ₉₀ (min)	D value (min)
UV	151 ± 41	169 ± 40
UV + Degussa P25 TiO ₂	198 ± 41	144 ± 5
UV + TiO ₂ –MWNTs nanocomposites	84 ± 29	72 ± 20

The current focus is to optimize the synthesis conditions for optimal performance and to combine all multi-functions into one particle. Tasks include (1) combining the modified titania shown in Figure 3 with the multifunctional material shown in Figure 2; (2) controlling the shape and size of silver nano-platelet for enhanced Raman sensing (for microbes and organic contaminants) and then adding the optimal silver onto materials mentioned above. The novel product will allow contaminant sensing, efficient water treatment, low equivalent system mass and easy regeneration in microgravity environment.

Development of a disposable microbial sensor for water quality monitoring based on a generic biosensor platform

Hyoung Jin Cho (University of Central Florida)

The objective of the proposed research is the development of a disposable microbial sensor for water quality monitoring based on a generic biosensor platform.

First, a subsystem, which serves as a generic biosensor platform, was developed in this project. Amperometric sensor electrodes, temperature sensors, fluidic access port, electrical connection pads, and microfluidic channels were designed in the generic biosensor platform. Then, based on the platform, a disposable microbial sensor for BOD (biochemical oxygen demand) measurement was realized.

The project contributes to the sensor development for space mission as well as ground applications by a generic platform based approach and by realizing low-cost disposable biosensor elements.

Design of Generic Biosensor Platform

A generic biosensor platform consisted of two layers; top microfluidic layer including channels and containers and bottom sensing layer including sensing electrodes. Figure 4 shows both the layers, these layers are held together by special medical grade adhesive films.

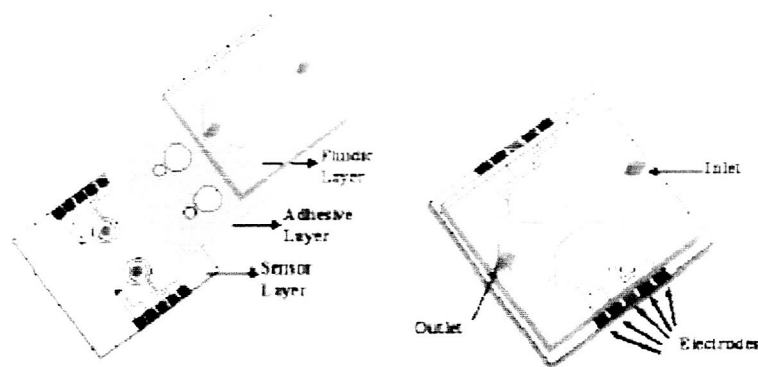


Figure 4. Schematic view of generic biosensor platform.

The top fluidic layer is micromachined by injection molding. A chemically inert, low cost, rugged COC (cyclic olefin copolymer) material is chosen for the fluidic layer. The top fluidic layer houses microfluidic channels, fluidic containers and injection ports as shown in Figure 5a. The fluidic channels are simple passive channels. The design allows sample solution to be introduced continuously into the sensor electrodes by external pump. The dimensions of the fluidic channel and the container are as shown in Figure 5b. The channel is $600\text{ }\mu\text{m}$ width x $340\text{ }\mu\text{m}$ depth, the chamber is $4500\text{ }\mu\text{m}$ width x $9400\text{ }\mu\text{m}$ length x $600\text{ }\mu\text{m}$ deep, and the approximate volume is about $200\text{ }\mu\text{L}$.

For a smooth flow of the analyte, an elliptical structure for the fluidic container has been chosen. The injection ports, inlet and outlet are designed to include standard size tubing of 1/16". Microfluidic channels and containers were fabricated on one side of the layer while tubings were made on the other side.

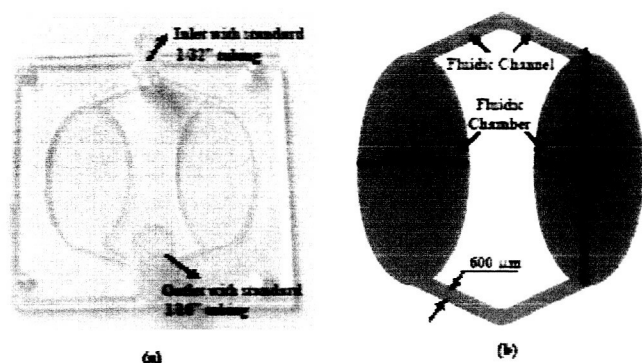


Figure 5. (a) Injection molded top view of the fluidic layer. (b) Schematic representation of the bottom view of fluidic layer with dimensions.

The sensor electrode layer also uses COC as a substrate. COC is choice over silicone or glass due to its low cost. In addition, it's chemically inert and optically transparent. Thus, it can be used for future integration of on-chip optical sensors. Sensor layer consisted of an amperometric sensor electrode and a temperature sensor to account for any thermal effects.

Amperometric Sensor: The designed amperometric sensor is a miniaturized electrochemical cell. The planar device is realized with a three electrodes design and can be used to determine the concentration of a wide range of redox active chemical species. The three terminals are RE (reference electrode), WE (working electrode), and CE (counter electrode). Though two terminal electrodes are simple to fabricate, they are not linear at upper ranges and have a slow response time. The principle of amperometry is based on the measurement of the current between WE and CE, which is induced by a redox reaction at WE.

Temperature Sensor: A gold meander type resistive type temperature sensor was designed alongside with the amperometric sensor. This is based on the temperature coefficient of resistivity of metals. Some metals have a very predictable change of resistance for a given change of temperature; these are the metals that are most commonly chosen for fabricating an RTS (resistance temperature sensors). A precision resistor is made from one of these metals to a nominal ohmic value at a specified temperature. By measuring its resistance at some unknown temperature and comparing this value to the resistor's nominal value, the change in resistance is determined. Since the temperature vs. resistance characteristics are also known, the change in temperature from the point initially specified can be calculated. We now have a practical temperature sensor, which in its bare form (the resistor) is commonly referred to as a resistance element. Platinum is by far the most popular due to its near linearity with temperature, wide temperature operating range, and superior long-term stability. Other materials are gold, nickel, copper, balco (an iron-nickel alloy), tungsten, and iridium.

In our application gold RTS was chosen because it could be patterned easier than platinum and formed simultaneously with the amperometric sensor electrodes. The operating range in our application is between 20° - 80° C. in this operating range gold behaves similar to platinum.

Design of a Microbial Sensor: The design of microbial sensors can be built upon the generic biosensor platform developed. First, by introducing an oxygen (gas, in generic terms) permeable membrane the generic sensor can be adopted to measure dissolved oxygen in water. Then, the biological component, i.e., immobilized microorganisms (microbial strain) covering the electrode area along with oxygen permeable membrane can be used to measure BOD (biochemical oxygen demand) of water. BOD determination with microbial sensors results from direct measurement of the oxygen consumption of microorganisms at the oxygen electrode. This consumption depends on the organic substrates that the microorganisms can utilize in the sample. The oxygen diffusing through the immobilized microorganism (and through the oxygen permeable layer) will get reduced due to respiratory activities of the microorganisms at the cathode, yielding a reduced current. Under steady state, the comparison between the dissolved oxygen level (without microorganism) and the partially consumed level (with microorganism) would give us information about aerobic activities of the microorganism, reflecting the BOD values. In our design, the generic biosensor platform can be coated with oxygen permeable layer for a dissolved oxygen sensor. Immobilizing the microorganisms over this would yield the BOD sensor.

Chlorine Sensor Potentiostatic Polarization: Once the reduction potential of free chlorine was established by cyclic polarization, potentiostatic polarization experiments were performed on the various samples. Figure 6 shows an example potentiostatic polarization on sample with 4ppm OCl^- ions. The figure shows that an initial settling time of about 20 seconds was required before stable output was observed. Since the electrochemical sensor follows the principle of amperometry the observed current is a function of the analyte (in this case OCl^- ions). Potentiostatic polarization is performed for all samples. The stable current output from all the potentiostatic polarization experiments were tabulated and plotted against the sample concentration. Figure 7 shows this plot. The sensor shows a good linear response in the range of 1.5 ppm – 8 ppm.

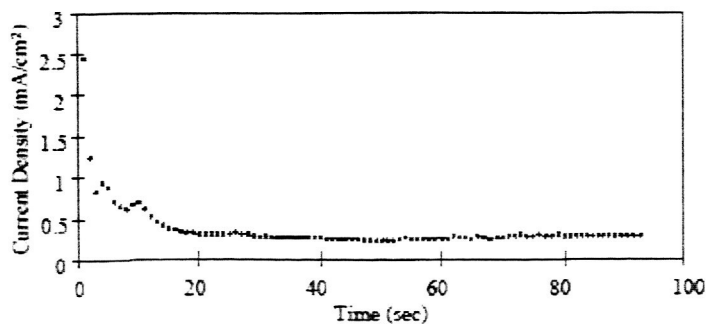


Figure 6. Time variation of sensor output measured in a solution with 4.0 ppm chlorine.

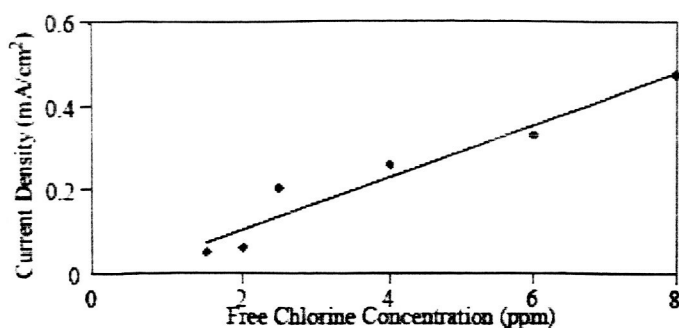


Figure 7. Sensor output as a function of free chlorine concentration.

Summary: We designed and fabricated a generic biosensor platform. The sensor was applied for the measurement of free chlorine in water samples. The results were compared with chemically measured values from HACH spectrophotometer and DPD (N, N-diethyl-p-phenylenediamine) ferrous titrimetric method. The sensor showed linear response to the variation in free chlorine concentration ranging between 1.5 ppm – 8.0 ppm at pH 9.

By using the generic biosensor platform, a flow-through microbial sensor was designed, fabricated and tested for rapid BOD measurements. The sensor was composed of two layers; the bottom layer containing ring-type amperometric sensor electrodes on a transparent cyclic olefin copolymer substrate and the injection-molded microfluidic layer on top. Fixed amount of microorganisms was immobilized over one sensor while the other bare sensor was used as the reference. The sensor was tested first with standard BOD solutions and then with actual wastewater samples collected from EWRF (from Eastern Wastewater Reclamation Facilities (EWRF) in Orange County, Florida. For comparison, standard BOD5 test was conducted. The sensor BOD measured within 20 minutes showed good agreement with BOD5 values measured over 5-day period up to 200 ppm of BOD. The developed sensor has numerous advantages including small size, low cost, quick response time, low sample volume requirement, and flexible design for various biochemical analyses.

Technical Advances—Updated Patents and Publications

Patents:

Provisional patent application Filed: Steve Horowitz, Mark Sheplak, Toshikazu Nishida, Louis Cattafesta (UF), Ken Tedjojuwono (NASA), "Floating-element shear-stress sensor using optical Moire' transduction technique," filed November 18, 2004.

Patent Application Filed: Toshi Nishida, Lou Cattafesta, Mark Sheplak, and Khai D.T. Ngo, "Resonant Energy MEMS Array Processor," United States Patent Application Number 10/436,534, filed 5/13/03.

Additional publications:

Mehta Anjum, Halakatti Shekhar, Seung H. Hyun and Hyoung J. Cho, "A Disposable Microbial Sensor for Rapid BOD Measurement", The 8th International Conference on Miniaturised Systems for Chemistry and Life Science (micro-TAS 2004), Malmo, Sweden, Sept. 26-30, 2004.

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Maneeratana, V., Bach, M., Mazyck, D. W., Wu, C. Y., Powers, K. and Sigmund, W. M., "Synthesis and Evaluation of Activated Carbon Composite Photocatalysts for Surface Enhanced Raman Scattering: Photocatalytic Layer Coating", *Proc. 34th Int. Conf. Env. Sys.*, No. 2004-01-2332, Colorado Springs, CO, July 19-22, 2004.

Lee, S.W., Drwiega, J., Wu, C. Y., Mazyck, D. and Sigmund, W. "Anatase TiO₂ Nanoparticle Coating on Barium Ferrite Using Titanium Bis-Ammonium Lactate Dihydroxide and Its Use as a Magnetic Photocatalyst", *Chemistry of Materials*, 16(6), 1160-1164, 2004 .

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Commercial Applications for Space Biotechnology

Principal Investigator: William Sheehan

Co-Investigators: Kristen Riley, Jean Andino, David Mazyck, Art Teixeira.

Project Goal and Anticipated Result:

The purpose of Commercial Applications Program was to identify and pursue commercial applications for NASA-related biotechnologies that offered the opportunity for stimulating growth in the biotechnology industry in the state of Florida, and elsewhere. Such NASA sponsored technologies included (1) biotechnologies specifically funded under the grant, (2) other UF biotechnologies having direct application to the NASA Space Biotechnology Program but funded by other sources, and (3) other NASA sponsored biotechnologies developed outside of UF where success in developing commercial applications was likely but not currently being pursued. The first option for pursuing commercial applications was to include industry partners on or about the time of proof of concept so that they would not only co-invest in the technology but also introduce the technology to the commercial market. Other technology transfer and commercialization options such as spin-offs or business incubations were also considered. Timely identification of commercial applications for developing technologies required commercial assessments, networking, and industry outreach to determine the appropriate match between the developing technology and the commercial partners.

Projects sponsored under the grant produced significant results that are evident in the six patent applications generated, two start-up companies initiated, and four licenses currently in-work. In support of these results, nine commercial assessments were performed and two in-depth market assessments. Several companies were contacted concerning collaboration and licensing opportunities. Results detailing these activities are addressed below.

Results:

(1) Biotechnologies Specifically Funded Under the Grant

Projects sponsored under the grant in the areas of Integrated Smart Nanosensors for Space Biotechnology Applications, and Space Agriculture and Biotechnology Research and Education (SABRE) produced significant results that are detailed below.

- Integrated Smart Nanosensors for Space Biotechnology Applications
 - o *Self Powered Health Monitoring Technology*

The subject technology is a microelectromechanical (MEMS) vibration energy harvesting device with corresponding control electronics that provide power for MEMS devices, such as pressure flow rate sensors. Indications from industry show that while competing research and commercialization is being done in harvested energy for MEMS electronics, there is a great need for

technology that produces more power in an inexpensive and robust package. This technology is technically significant, novel and commercially important. A commercial assessment and an in-depth marketing assessment were performed on this technology. The researchers are in the process of reviewing the market assessment results. A patent application was filed on this technology.

- *Miniature Low-Power Integrated CO/CO₂/H₂/H₂O/O₂ Sensor for Space Biotechnology Applications*

The subject invention is a high temperature, solid-state, electrochemical sensor to detect gaseous CO, CO₂, H₂, H₂O, NO_x, and O₂. These gases are detected when they come into contact with the sensor's electrodes. The sensor in turn produces a millivolt signal which indicates the concentration of the gas. The new sensor technology offers numerous advantages over current gas sensing technologies, including versatility, size, manufacturing technique, cost, simplicity, low power requirement, and high temperature operation. Initial market research indicates a large market for gas sensors, especially for one having multiple sensors on one chip. This technology is considered to be an evolutionary improvement over what is currently available. The technology has application in power plant use, automobiles, and commercial safety devices. A commercial assessment was performed on this technology and a patent application filed. The researcher has started a company by the name of Solid State Ionic Devices, and is in preliminary license negotiations with the University Office of Technology Licensing.

- *Wide Energy Bandgap Based Gas Sensors*

The subject invention uses W and WSix contacts for high quality ohmic and Schottky contacts deposited on GaN, with a view to stable operation up to 600 C. These GaN Schottky diodes are sensitive to various gases, detect these gases up to temperature of at least 600 C, have 1-ppm level detection of gas in less than 1 second, and signal strength of 10 mA and 0.5 V. Results of a preliminary commercial assessment show that pursuing a patent on the technology will be extremely difficult due to past publications describing the technology. However, commercial applications may exist in hydrogen gas feed control and leak detection in fuel cells, and in the monitoring of emissions from combustion systems.

- Space Agriculture and Biotechnology Research and Education (SABRE)

- *Enhancing starch production in plants*

The subject invention is a method to increase the starch content of plants such as corn, potatoes, and wheat. This invention increases starch production through the use of proteins which in turn control enzymes that regulate starch production. The inventors have discovered that starch production in plants is enhanced relative to levels observed in wild or control plants by reducing the activity of plant 14-3-3 proteins. This allows the starch content of the plant to increase two to four times higher than that found in a natural plant. This invention has also been found to increase the branched glucan content of starch produced by plants.

This invention also offers an additional level of control of starch production in plants over the existing art. The invention can be used in three main markets: foods, energy, and biopolymers.

All genetically modified crops must be approved by the United States Department of Agriculture prior to commercialization. In addition to USDA approval, any GM crop that is to be used in the food chain must also be approved by the FDA. Since the regulatory approvals for the use of the subject invention in the energy market is likely to be easier than that required for use in the food market, initial marketing activities started in this market. A commercial assessment and an in-depth market assessment were performed on this technology. A patent application has been filed and an exclusive license has been negotiated between the University Office of Technology Licensing and United Kingdom company PBL.

- *Genomic Targets of Green Light Effects on Plant Structure*

The subject technology is in the early stages of development, where plants grown in green light showed an increase in stem growth compared to plants grown in darkness. The potential exists to create plants that show preferential growth characteristics by modifying one or more of the genes identified in the differential display analysis. At this time, however, 600 genes were altered in response to green light. Which ones are specific for the green light growth affects is still yet to be determined. Additional research is needed to identify them. A commercial assessment was performed on this technology.

- *Development of a genetics-based computer model for the engineering of new crops*

The subject invention is a modification of crop simulation software to include genetic coefficients as predictors for crop outcomes. The invention inserts state variables for seed size, pod size, pod dry matter accumulation, and seeds per pod to allow for the design of new crops with these attributes. This technology may have possible application to agricultural seed and pharmaceutical companies, farm managers, crop consultants, and environmental agencies for engineered crops, seasonal and locational growth prediction, and decisions for water and land management. Results of a preliminary commercial assessment indicate that although agricultural simulation models have been in existence for 15 to 20 years, technology adoption has been poor outside of the research realm. As such, a patent application is not being pursued and the researchers will share the technology with all interested parties.

- *Novel Support Matrices for Immobilization of Anaerobic Consortia*

The subject invention is a set of matrix materials selected to enhance the anaerobic treatment and digestion of waste in a bioreactor. The subject technology encompasses a range of materials with varying composition, porosity, surface texture, and surface chemistry that are intended to encourage attachment of the anaerobic bacteria and regulate their spatial distribution.

In laboratory tests, the use of the matrix material for immobilization of an anaerobic consortium in the treatment of fatty acids increased the levels of weekly methane production by 30% and 200%, as compared to the non-matrix controls, after 1 and 2 weeks, respectively. Results of a preliminary commercial assessment indicate that additional research is required to better specify descriptions of the preferred matrix materials. Interest from multiple companies has been noted, and they wish to be kept informed as the technology progresses. A patent application has been filed on this technology.

(2) University of Florida Biotechnologies Having Direct Application to the NASA Space Biotechnology Program But Funded by Other Sources

Method and Apparatus for Purifying Flue Gases from Combustion Sources

The subject invention is a spin-off of a NASA ES CSTC funded technology titled "Magnetically Agitated Photocatalytic Reactor" for use in space water remediation. This spin-off technology is novel for removing elemental mercury vapor from flue gas. It offers several advantages over existing technologies by having a greater than 99% removal efficiency, greater capacity, low material-to-pollutant ratio leading to as much as 90% reduction in the volume of material required, lower power consumption, cost effective, and saleable fly ash. Results of a preliminary commercial assessment showed great interest in the technology and large commercial opportunity. EPA regulations on emissions will be tighter in the near future, and this technology may meet the need. A commercial assessment was performed on this technology and a patent application filed. A start-up company by the name of Sol-Gel Power Technologies is based on this new technology, and they are in preliminary license negotiations with the University Office of Technology Licensing.

Flooded Densified Leachbed Anaerobic Digestion

The subject invention is a new version of the patented high-solids process sequential batch anaerobic composting (SEBAC) system which has been modified to operate under hypo- and micro-gravity environments of space missions. These process modifications reduce reactor size and accommodate microgravity with flooded leachbeds with forced pumping to avoid the need for gravity, external gas liquid separators, and densification of biomass in the leachbeds. A laboratory system was developed to test these modifications which led to the design of a full-scale prototype system designed to process wastes from a 6-person crew during a Mars mission. Performance of the laboratory system showed significant improvement over the non-flooded conventional mode of SEBAC operation. In fact a doubling of the kinetics of conversion was observed for the space SEBAC at an operation temperature 35°C compared to 50°C used for the conventional system. The flooded operation resulted in an approximate 2X improvement in conversion kinetics. This occurred in spite of the 15°C lower operating temperature. The second improvement is strictly mechanical, i.e. densification allows a 3X increase in feed to reactor volume ratio which translates to a smaller reactor size. A commercial assessment was performed on the original SEBAC technology, with several terrestrial applications being identified in the food processing, animal waste, wood-working industries. This new technology is applicable to these same industries.

A patent application has been filed. In addition, the University received a \$1 M Grant from Xcel Energy to put in a pilot facility at the American Crystal Sugar facility in East Grand Forks Minnesota over the next three years to process sugar beet tailings.

(3) NASA Sponsored Biotechnologies Developed Outside of the University of Florida, Where Success in Developing Commercial Applications is Likely but Not Currently Being Pursued

Process for Producing Vegetative and Tuber Growth Regulator

The subject invention is a process of making a vegetative and tuber growth regulator for potatoes. It results in a tuber-inducing factor (TIF) that accelerates tuber formation and reduces the vegetative growth. Advantages of this technology include a 15% to 20% increase in seed production, a 20% decrease in the time to harvest, a significant reduction in excess shoot growth, and a reduction in production costs associated with nutrient replenishment, waste water treatment and disposal. Tuber initiation typically occurs 7 to 10 days earlier and plant height is reduced by 50% to 80%, with harvest index increasing from 60% to 80%. This technology was developed under a Cooperative Agreement between NASA Kennedy Space Center and Dynamac Corporation. Dynamac owns the rights to this technology and is interested in affiliating it with this section of the Space Biotech Grant for commercialization purposes. Discussions have taken place and an agreement has been executed as a condition of the grant to provide a potential downstream return on investment for the UF-NASA biotechnology program. A commercial assessment was performed on this technology, and a research license is currently in-work between Dynamac and the Maine Seed Potato Board. A potential collaboration is also in work with the Scottish Crop Research Institute.